

Disease Notes

First Report of Pyrimethanil Resistance in *Botrytis cinerea* from Stored Apples in Pennsylvania

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Botrytis cinerea Pers.: Fr. (teleomorph *Botryotinia fuckeliana* [de Bary] Whetzel) causes gray mold on apple fruit. A survey of commercial packinghouses in Washington State revealed that it accounted for 28% of the decay in storage (1). Fungicide application coupled with cultural practices are the primary method of control as all commercial apple cultivars are susceptible to gray mold. In February 2013, gray mold was observed at ~5% incidence for commercially packed 'Gala' apple fruit that had been treated with Penbotec (active ingredient: pyrimethanil, Shield-Bright, Pace International) prior to controlled atmosphere storage in Pennsylvania. Eight infected apple fruit were collected, placed in 80 count boxes on cardboard trays, and stored at 4°C. One isolate was obtained from each decayed apple, placed on potato dextrose agar (PDA) petri plates, and incubated at 20°C with natural light. Eight single-spore isolates were identified as *B. cinerea* based on cultural characteristics. Species level identification was executed by obtaining mycelial genomic DNA, amplifying the ITS rDNA, and sequencing the ~550-bp amplicon directly (2). MegaBLAST analysis of the 2X consensus for the 8 isolates revealed 100% identity to *B. cinerea* ITS sequences in GenBank (KF156296.1 and JX867227.1) with E values of 0.0, thus confirming the morphological identification. Minimum inhibitory concentration (MIC) was determined using conidial suspensions obtained from ~14-day-old plates (10^4 spores/ml) and a range (0 to 500 µg/ml) of technical grade pyrimethanil on three replicated 96-well microtiter plates containing a defined medium for each experiment. Conidial proliferation was inhibited at 250 µg/ml for all eight isolates and the experiment was conducted four times. To further define the resistance levels between the isolates, mycelial growth analysis using a plug of actively growing mycelium from the margin of ~3-day-old plates was conducted with a defined medium three times with technical grade pyrimethanil with three plates per experiment. Five isolates grew at 250 µg/ml (highly resistant), while three did not (moderately resistant). To assess resistance in vivo, organic 'Gala' apples were rinsed with soap and water, sprayed with 70% ethanol, placed on trays, and allowed to air dry. Apples were wounded with a sterile finishing nail, inoculated with 20 µl of a conidial suspension (10^4 spores/ml) of either a moderately or a highly resistant isolate, and dipped in the labeled application rate of Penbotec at 500 µg/ml or sterile water for 30 s. Fruit were stored in 100 count boxes at 22°C for 5 days and decay incidence and severity were recorded. Ten fruit composed a replicate per treatment and the experiment was repeated. Water inoculated controls were symptomless and water-dipped inoculated fruit had 100% decay. Penbotec-treated fruit had 100% decay incidence and mean lesion diameters of 37.6 (±13.1 mm) for the highly, and 35.7 (±9.0 mm) for the moderately resistant isolate. This is the first report of pyrimethanil resistance in *B. cinerea* from decayed apples collected from a commercial packinghouse in Pennsylvania. The results indicate that pyrimethanil resistance has developed in *B. cinerea*, which can result in control failures on Penbotec-treated fruit during storage. Furthermore, it emphasizes the need for additional tools to manage gray mold on apple fruit and may pose issues for export concerning the spread of fungicide-resistant inoculum.

References: (1) Y.-K. Kim and C. L. Xiao. Plant Dis. 92:940, 2008. (2) T. J. White et al. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA, 1990.